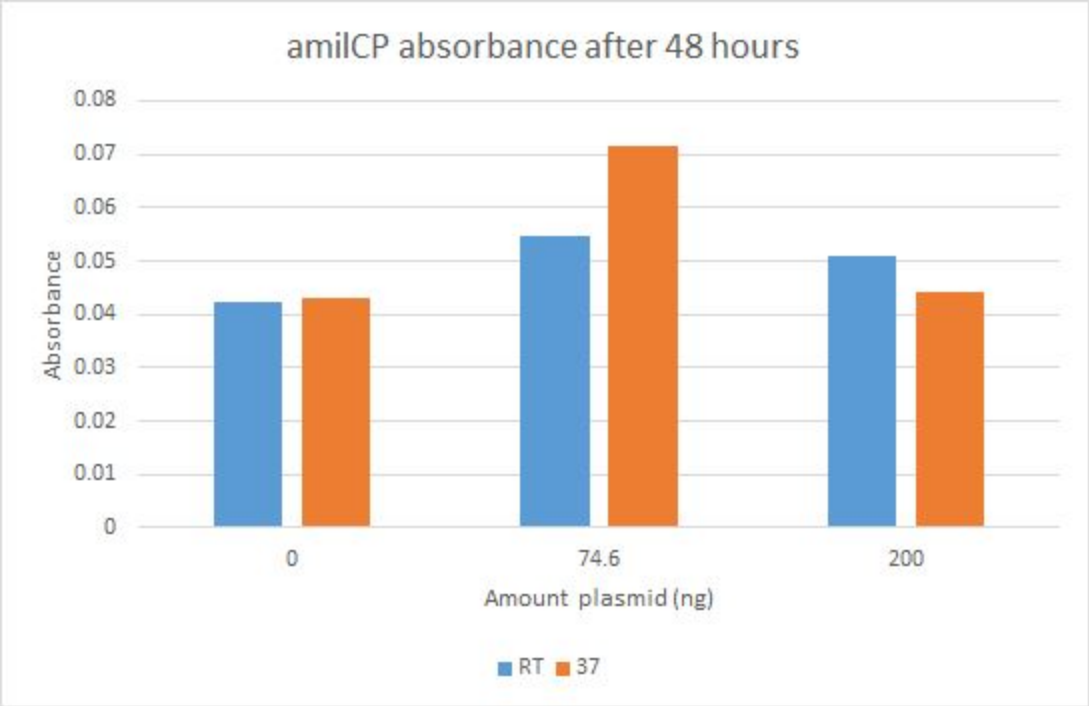
iGEM 2016: Pittsburgh
Week 7 Lab Notebook

Tuesday, July 5

Lab meeting
Resuspend DNA oligos in water at 100 uM
lacZ agar stab tube into incubator
Weekend amilCP readings (no visible color)

Amt plasmid (ng)	RT (D)	37 (C)
0 (10)	0.0425	0.0429
74.6 (11)	0.0547	0.0714
200 (12)	0.051	0.0443



Wednesday, July 6

No lacZ growth
Anneal oligos (Claire)

P Strand_Collins D	DNAzyme_Collins D
PS Substrate_DNAzyme D (Ref No. 90)	DNAzyme D Collins Edit
P Strand_Collins G	DNAzyme_Collins G
PS Substrate DNAzyme G (Ref No. 92)	DNAzyme G Collins Edit

50 nM : 50 nM 1 uL 2.5 uM substrate

1 uL 2.5 uM DNAzyme
5 uL 10X T4 DNA Ligase Buffer
43 uL water

50 nM : 75 nM
1 uL 2.5 uM substrate
1.5 uL 2.5 uM DNAzyme
5 uL 10X T4 DNA Ligase Buffer
42.5 uL water

50 nM : 100 nM
1 uL 2.5 uM substrate
2 uL 2.5 uM DNAzyme
5 uL 10X T4 DNA Ligase Buffer
42 uL water

Cell-free T7-GFP in 384 well plate (Claire)

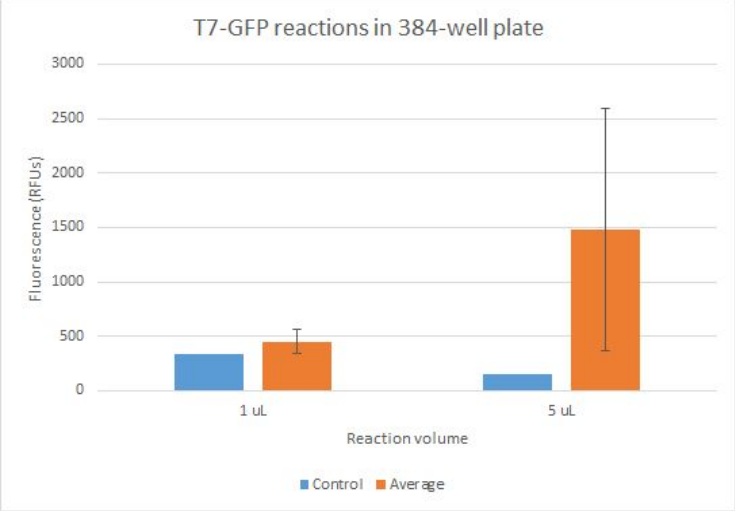
	5 uL (x2)	5 uL control	1 uL (x2)	1 uL control
Solution A	2	2	0.4	0.4
Solution B	1.5	1.5	0.3	0.3
RNAse inhibitor	0.05	0.05	0.01	0.01
water	1.243	1.45	0.25	0.29
DNA (5 ng/uL)	0.207	0	0.04	0

Master mix: 7.2 uL Solution A
5.4 uL Solution B
0.18 uL RNAse inhibitor

Remove 3.55 uL for 5 uL control // 0.71 uL for 1 uL control
Add 1.45 uL water // 0.29 uL water

To master mix, add 0.404 uL DNA and 2.986 uL water

Separate into 2 x 5 uL and 2 x 1 uL



	Control	1	2	Average	st dev
1 uL	337	530	371	450.5	112.43
5 uL	153	692	2264	1478	1111.572

Thursday, July 7

Re-check amilCP-terminator ligations (Claire)

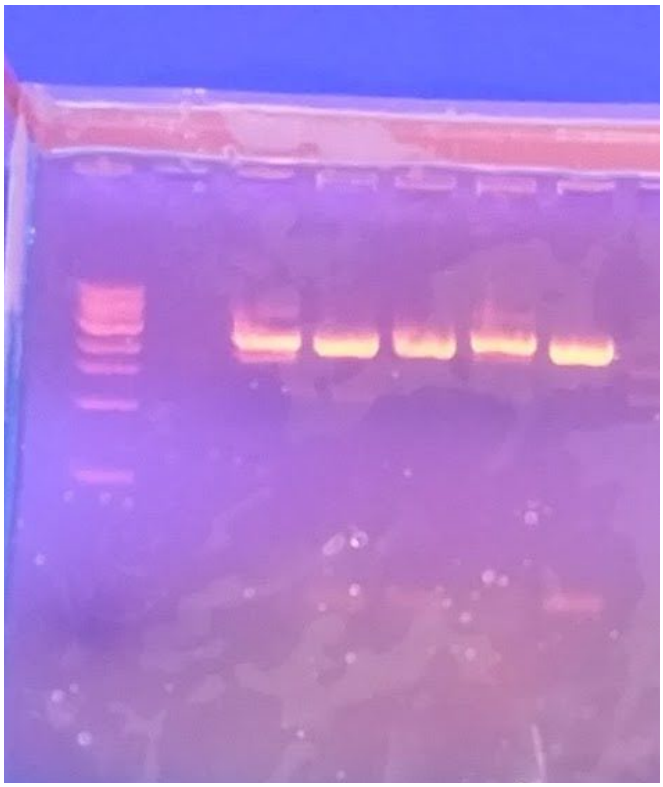
Sequence suggests empty plasmid

Digest 0.5 uL Eco RI
0.5 uL PstI
1 ug DNA = 6.19 uL amilCP 1
6.98 uL amilCP 2
Out of original; from 6/29 6.70 uL amilCP 3
6.71 uL amilCP 4
7.25 uL amilCP 5
From 6/30 16.03 uL amilCP 8
1 uL buffer
H2O = 1.81 uL amilCP 1
1.02 uL amilCP 2
1.30 uL amilCP 3
1.29 uL amilCP 4
0.75 uL amilCP 5
0.00 uL amilCP 8

Gel lane 1 ladder
2 uncut amilCP 2 plasmid
3 1
4 2
5 3
6 4
7 5
8 8

Nothing correct

Add Jason T7 cells to old amilCP 3 tube (should have been correct) and transform



Cell-free Collins plasmids with annealed oligos (Maddie)

Dilute all DNA to 50 nM

D switch

	Control A 1-2	Mis-Match A 3-4	DNA trigger A 5-6	Duplex 1:1 A 7-8	Duplex 1:1.5 A 9-10	Duplex 1:2 A 11-12	PO strand A 13-14	Enzymatic strand A 15-16
Sol'n A	2	2	2	2	2	2	2	2
Sol'n B	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
substrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
switch	0.6	0.6	0.6	0.6	0.6	0.6	0.6	0.6
trigger	0	0.4	0.4	0.4	0.4	0.4	0.4	0.4
water	0.4	0	0	0	0	0	0	0

G switch

	Control B 1-2	Mis-Match B 3-4	DNA trigger B 5-6	Duplex 1:1 B 7-8	Duplex 1:1.5 B 9-10	Duplex 1:2 B 11-12	PO strand B 13-14	Enzymatic strand B 15-16
Sol'n A	2	2	2	2	2	2	2	2
Sol'n B	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
RNAse inhibitor	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
substrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
switch	0.52	0.52	0.52	0.52	0.52	0.52	0.52	0.52
trigger	0	0.4	0.4	0.4	0.4	0.4	0.4	0.4
water	0.48	0.08	0.08	0.08	0.08	0.08	0.08	0.08

32 reactions

Master (34 rxns): 68 uL Solution A
51 uL Solution B
8.5 uL RNAse inhibitor
8.5 uL substrate

Separate into 2 x 136 uL

D // G

Add 9.6 uL // 8.32 uL switch DNA

Remove 9.2 uL // 9.04 uL for controls

Add 0.8 uL // 0.96 uL water

2 CONTROLS DONE

Add 1.2 uL water

Separate into 7 x 9.2 uL

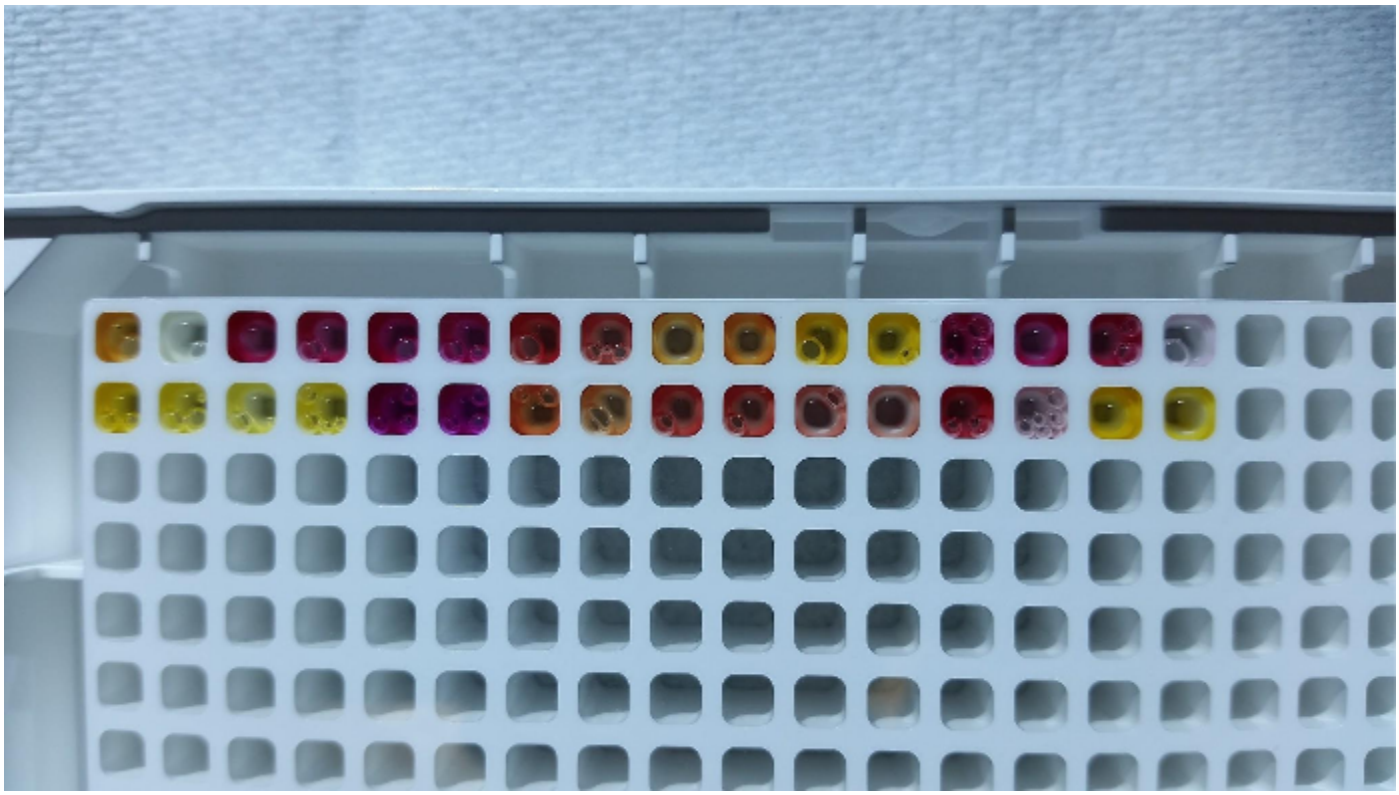
Add 0.8 uL appropriate DNA

Plate 5 uL duplicates, read at 570 nm, 384 well plate

Plate01

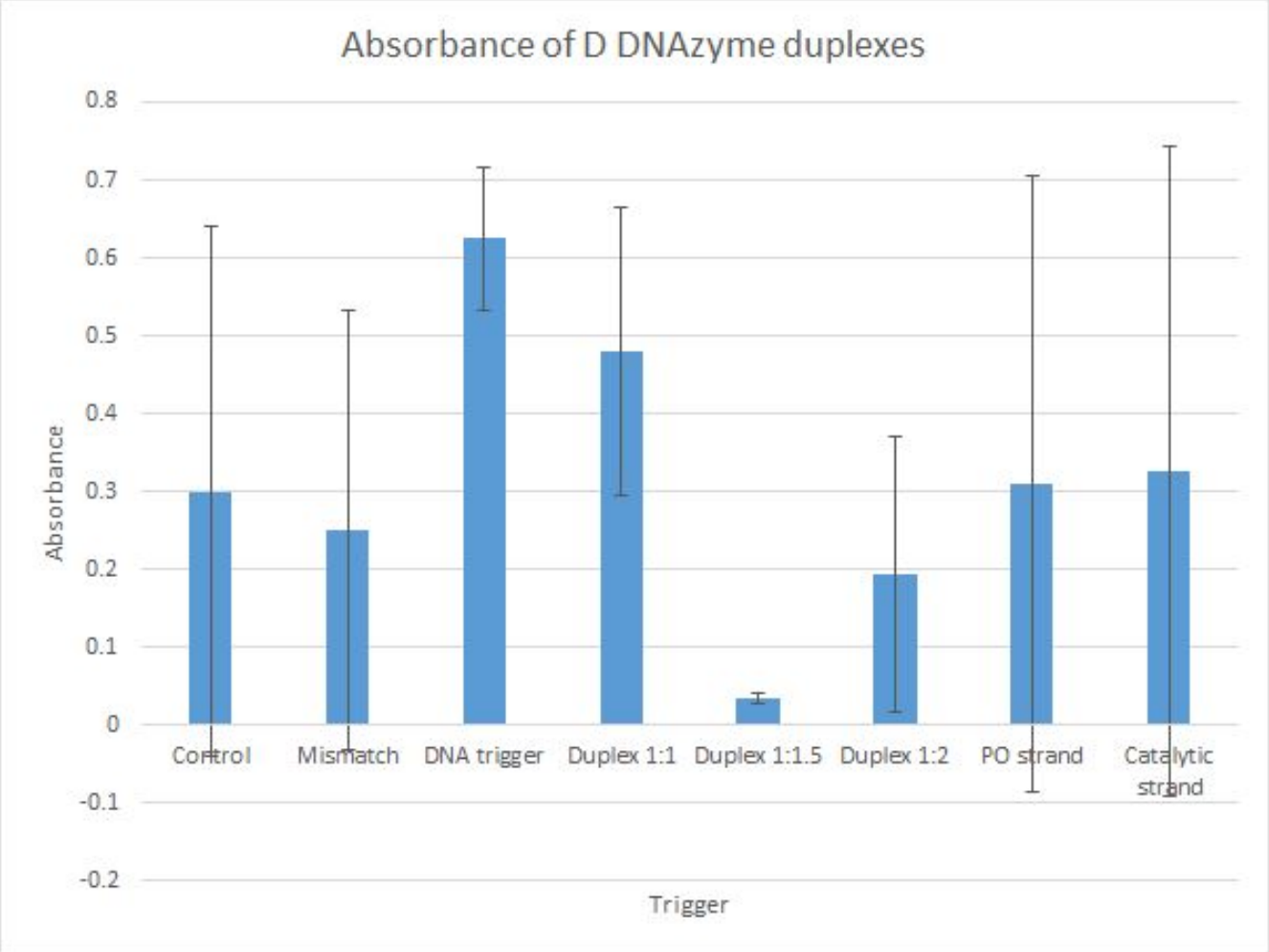
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
A	0.54	0.06	0.05	0.45	0.69	0.56	0.61	0.35	0.03	0.04	0.32	0.07	0.59	0.03	0.62	0.03
B	0.21	0.50	0.03	0.09	1.48	1.22	0.35	0.27	0.30	0.23	0.13	0.03	0.49	0.03	0.05	0.03

Dilute to 10 uL total

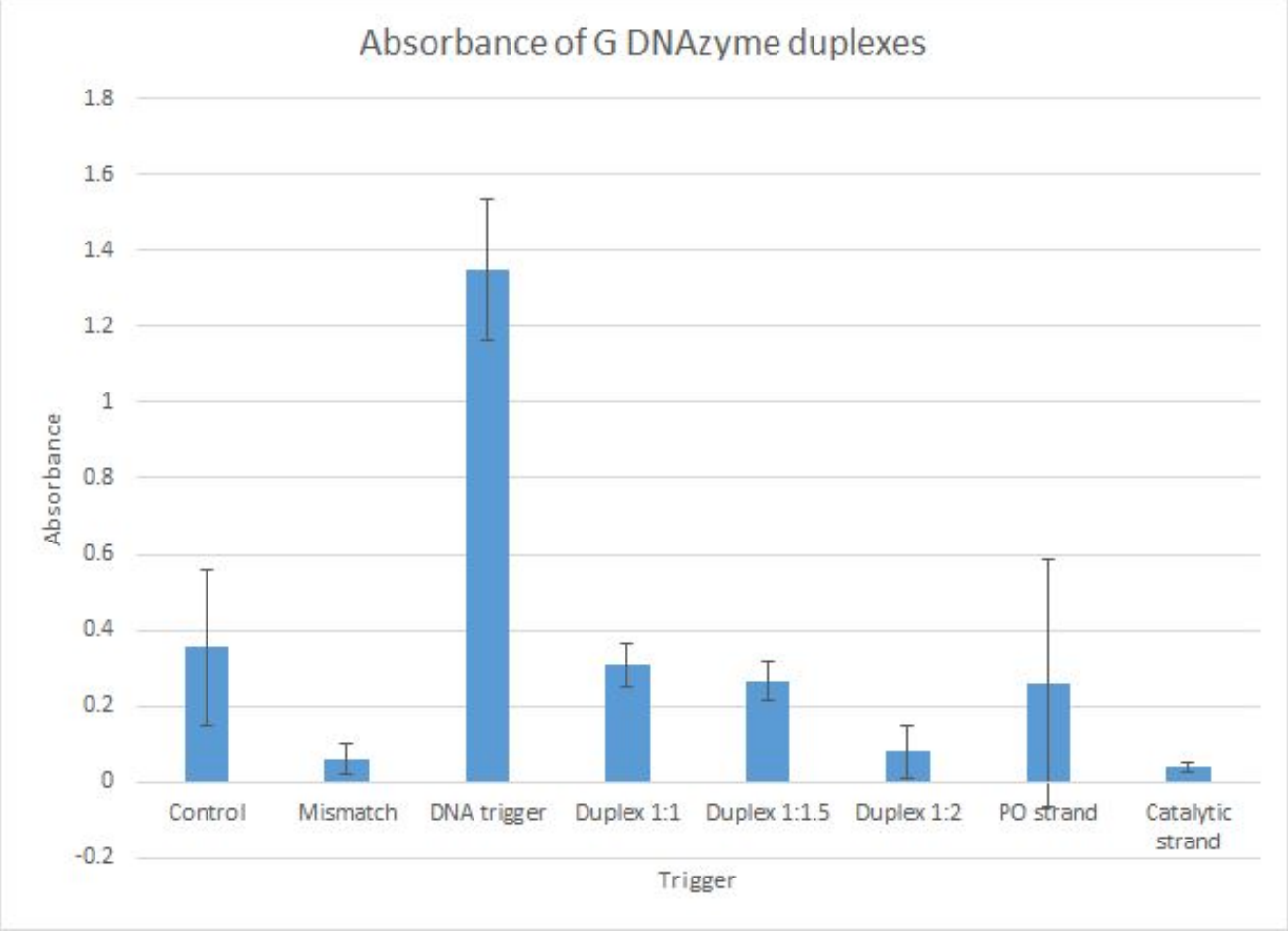


RESULTS

D switch	Absorbance 1	Absorbance 2	Average	St Dev
Control	0.54	0.06	0.3	0.339411
Mismatch	0.05	0.45	0.25	0.282843
DNA trigger	0.69	0.56	0.625	0.091924
Duplex 1:1	0.61	0.35	0.48	0.183848
Duplex 1:1.5	0.03	0.04	0.035	0.007071
Duplex 1:2	0.32	0.07	0.195	0.176777
PO strand	0.59	0.03	0.31	0.39598
Catalytic strand	0.62	0.03	0.325	0.417193



G switch	Absorbance 1	Absorbance 2	Average	St Dev
Control	0.21	0.5	0.355	0.205060967
Mismatch	0.03	0.09	0.06	0.042426407
DNA trigger	1.48	1.22	1.35	0.183847763
Duplex 1:1	0.35	0.27	0.31	0.056568542
Duplex 1:1.5	0.3	0.23	0.265	0.049497475
Duplex 1:2	0.13	0.03	0.08	0.070710678
PO strand	0.49	0.03	0.26	0.325269119
Catalytic strand	0.05	0.03	0.04	0.014142136



Friday, July 8

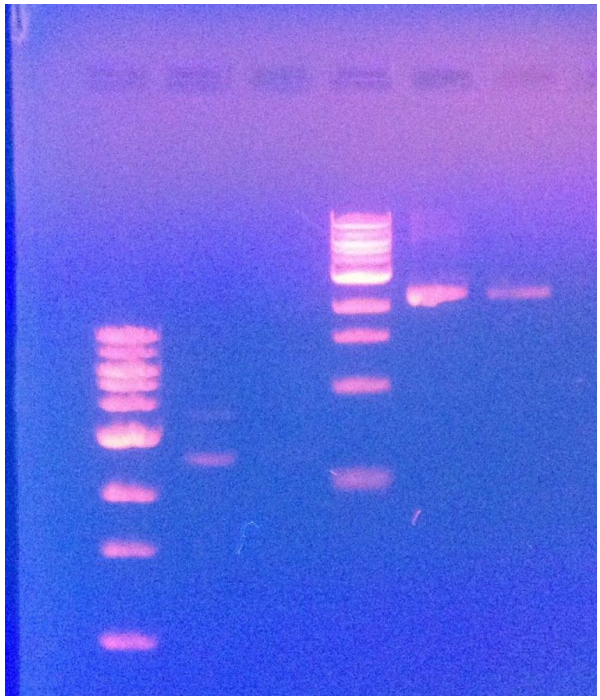
amilCP plate not blue

PCR of T7-eGFP-terminator construct (Claire)

50 uL reaction: 32.5 uL water
10 uL 5x Phusion buffer
1 uL dNTP's
2.5 uL 10 uM forward primer
2.5 uL 10 uM reverse primer
1 uL template (about 200 ng)
0.5 uL DNA polymerase

Gel check PCR (Claire) Lane 1/4: ladder
Lane 2/5: uncut plasmid
Lane 3/6: reaction

First run: couldn't see DNA clearly
Band should be about 800 bp
PCR did not work

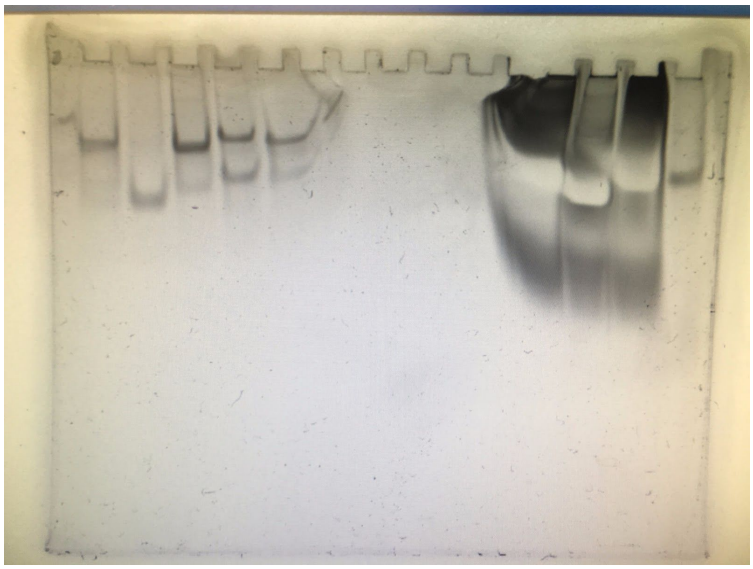


Anneal DNAzyme in presence of erbium

Tried to dissolve 1.909 g Er in 250 uL H2O
Ended up scooping paste with pipette tips
Very imprecise and uneven across rxns
50 nM : 75 nM
D old and edit; G old and edit (4)
1 uL 2.5 uM substrate
1.5 uL 2.5 uM enzymatic strand
5 uL buffer
25 uL (0.192 g) 2X Er
22.5 uL water
Incubate and cool

Test annealed oligos on PAGE gel: Native acrylamide gel lane (Maddie) (Nick @ Deiters)

1 Old G 1:1.5 oligo
2 Old D 1:1.5 oligo
3 Edited G 1:1 oligo
4 Edited D 1:1 oligo
5 Old G 1:1 oligo
6 Old D 1:1 oligo
7 Erbium rxn old G
8 Erbium rxn edited G
9 Erbium rxn old D
10 Erbium rxn edited D
11 Cat strand G



12 PO strand G
13 Cat strand D
14 PO strand D

Test annealed oligos on PAGE gel: Urea acrylamide gel lane (Maddie)

1 PO strand D
2 Cat strand D
3 PO strand G
4 Cat strand G
5 Erbium rxn old G
6 Erbium rxn edited G
7 Erbium rxn old D
8 Erbium rxn edited D
9 Old D 1:1 oligo
10 Old G 1:1 oligo
11 Edited D 1:1 oligo
12 Edited G 1:1 oligo
13 Old D 1:1.5 oligo
14 Old G 1:1.5 oligo

Erbium rxns didn't run → could be a charge issue

Nick recommends a buffer exchange

Not enough wells to run all of the oligos → can finish next week